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STUDIES OF THE METAL-ION BINDING CHARACTERISTICS OF SOLUBLE, NATURAL ORGANIC MATERIALS

A

DISSERTATION

Presented to the Faculty of the
University of Alaska in Partial Fulfillment
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for the Degree of
Doctor of Philosopy

By

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College, Alaska

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STUDIES OF THE METAL-ION BINDING CHARACTERISTICS

OF SOLUBLE, NATURAL ORGANIC MATERIALS

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ABSTRACT

A theory of multiple equilibria is presented which assumes the numerous binding sites on humic molecules as subject to competition between protons and metal ions for occupancy. The binding of small ions to active sites on the polymer is considered the result of electrostatic interactions which vary with the degree of humic ionization. This theory allows the calculation of intrinsic formation constants, which are independent of the degree of polymer dissociation, for humic-metal complexes. Thus, reliable estimates of the strengths of natural associations can be obtained.

Although this approach has been extensively employed in the study of protein-metal interactions, the existing equations have been modified to accomodate the lack of an average-molecular-weight value for the humic material.

The experimental determination of the intrinsic formation constants was accomplished by dialysis equilibration and Electron Spin Resonance spectroscopy(ESR). Although suitable for only Mn(11) determinations, ESR was found to provide a simple technique for the investigation of manganous complexes regardless of ligand complexity.

ACKNOWLEDGEMENTS

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SECTION 1

INTRODUCTION

Because of the increasing possibilities of heavy-metal pollution, an understanding of the aqueous chemistry of these metals has become a practical necessity rather than a geochemical or biological curiosity. Consequently, not only is information needed regarding the total dissolved concentrations of the major and minor constituents for a given natural system, but also thermodynamic constants are required for the various cation complexation reactions involving both inorganic and organic ligands. Employing published formation constants, Sillen (1961) and Zirino and Yamamoto (1972) have discussed the chemical composition of seawater from the aspect of ion-pair formation. However, both the experimental verification of the existence of organic complexes (Barsdate and Matson, 1966; Slowey, et al., 1967) and the anomalous behavior of some elements from that predicted by the ion-pair model (Sillen, 1961) indicate that the influence of soluble, natural organic materials (SNO) must be quantified by means of equilbrium equations if trace-metal cycling processes are to be correctly understood.

However, uncertainties are imposed upon the application of a thermodynamic treatment to natural water systems by the existence of equilibria which may be unsuspected or for which

quantitative activity data are not available. Hence, to effect an estimation of the strength of natural organic complexes, concentrations are substituted for activities in the required expressions. In addition, the difficulties encountered in mathematically describing the acid dissociation of SNO materials lead to a further reduction in rigor. These complications have resulted in the adoption of two classes of "improvised" formation constants: the "practical" formation constant and the "apparent" formation constant.

In general, the equilibrium reaction for complex formation can be written as

$$x M + y A \rightleftharpoons M_x A_y \qquad (1.1)$$

where M is the metal ion, A the complexing agent, and x and y the stoichiometric coefficients of the reactants. Charges have been deleted for simplicity.

The thermodynamic formation constant (K_T) for the complex is then,

$$K_T = \frac{(M_x A_y)}{(M)^x (A)^y} \cdot \frac{(\gamma_{M_x A_y})}{(\gamma_M)^x (\gamma_A)^y} \qquad (1.2)$$

When methods are not available for the determination of the activity coefficients (γ_i) of the individual species, the system is usually "swamped" by excess neutral salt to maintain the ionic strength at a constant value. Equation (1.2) then reduces to equation (1.3).

$$K_s = \frac{(M_x A_y)}{(M)^x (A)^y} \quad . \tag{1.3}$$

The formation constant (K_s) in equation (1.3) is termed the "stoichiometric" or "practical" formation constant.

The concentration of the bonding species (A) in equation (1.3) is defined as

$$(A) = C_A \alpha_A \tag{1.4}$$

where C_A is the total concentration of the complexing species present in solution and α_A is the fraction of the total concentration in the form suitable for binding metal ions. Substitution of equation (1.4) into (1.3) yields

$$K_s = \frac{(M_x A_y)}{(M)^x (C_A \alpha_A)^y} \quad . \tag{1.5}$$

The fraction α_A is, however, both a function of the system pH and the step-wise acid dissociation constants of the complexing agent; e.g. in a solution of oxalic acid, the fraction existing as the oxalate ion is

$$\alpha_{C_2O_4^{-2}} = \left[\frac{(H)^2}{K_1 K_2} + \frac{(H)}{K_1} + 1 \right] \quad . \tag{1.6}$$

Because neither the form of the SNO metal-binding species nor the organic step-wise dissociation constants are known, complexation experiments are performed at specific pH values in order that α_A is reduced to a constant for each determination. Thus, equation (1.6) may be rewritten and an

TABLE 1

Metal	(1) $\log K_f'$ pH 3.0	pH 5.0	(2) $\log K_{HOx}$
Cu^{+2}	3.3	4.0	12.2
Ni^{+2}	3.2	4.2	9.9
Co^{+2}	2.8	4.1	8.6
Pb^{+2}	2.7	4.0	9.0
Zn^{+2}	2.2	3.6	8.6
Mn^{+2}	2.2	3.7	6.8

Fulvic Acid Formation Constants Determined By The Ion-Exchange Technique

(1) Schnitzer and Kahn, (1972).

(2) Sillen and Martell, (1964).

actions occur have been demonstrated for a number of synthetic and biologically important polyelectrolytes (Arnold and Overbeek, 1950; Scatchard, et al., 1957).

In the following sections, a theory of multiple equilibria will be presented which considers the natural organic molecules as possessing numerous bonding sites, all of which are subject to the competitive equilibria between protons and metal ions for occupancy. Because the overall situation may be viewed as reactions between large molecules and simple ions, the interactions between sites and counterions have been considered to be purely electrostatic in nature.

Although it is obviously preferable to characterize the metal-ion binding of SNO materials by microscopic or step-wise formation constants than to employ the variable quantity K_f' of equation (1.7), experimental difficulties force the use of "apparent" formation constants to describe natural associations. However, an intrinsic formation constant, K_{Int}, which is independent of the degree of polyacid dissociation can be factored out of the "apparent" constant. The definition of the intrinsic formation constant requires K_{Int} for a given system to have close to the same magnitude as the formation constant describing the interaction between the metal-ion and a monomeric unit of the polymer. Thus, experimental K_{Int} values provide insight into the absolute strengths of SNO-metal asso-

ciations.

Although this approach has seen extensive use in the field of biochemistry (Tanford, 1967), the existing equations have modified (Section 3) to accomodate the lack of an average-molecular-weight value for the SNO ligand. In addition, detailed experimental procedures have been presented whereby the parameters required by the working expressions can be determined and the intrinsic formation constants in question, calculated.

SECTION 2

THE ACID DISSOCIATION OF SOLUBLE, NATURAL ORGANIC MATERIALS

Kodema and Schnitzer (1967) concluded from X-ray diffraction studies that because humic and fulvic acids possess no identifiable monomeric units, no two carboxyl groups are chemically identical. However, the question then arises as to the degree of dissimilarity between groups. If the acidities of the carboxylic groups do not vary appreciably over a narrow range, group homogeneity may be assumed and the observed pH dependent variations in SNO acidity explained by treating the ionization of the numerous, identical functional groups present on a SNO molecule as a random phenomena.

GENERAL EQUATIONS

The protonation of a completely dissociated high-molecular-weight polyacid (P^{n-}) may be represented by the following equations:

$$\begin{aligned} (HP) &= K_1 (H)(P) \\ (H_2P) &= K_1 K_2 (H)^2(P) \\ &\vdots \\ (H_nP) &= K_1 K_2 \cdots K_n (H)^n(P) \end{aligned} \qquad (2.1)$$

Although the experimental determination of the concentrations of the species present such as (HP), (H_2P), etc., is usually impossible, the average number of protons combined with each macromolecule can normally be determined by a number of techniques. This number is designated $\bar{\nu}$, and is

defined as

$$\bar{\nu}_H = \frac{\text{moles of bound proton}}{\text{moles of polymer}} \qquad (2.2)$$

For a given system, the mass balance equations for the proton and the polymer are, respectively, equations (2.3) and (2.4).

$$(HP) + 2(H_2P) + 3(H_3P) + \ldots + n(H_nP) \qquad (2.3)$$

$$(P) + (HP) + (H_2P) + \ldots + (H_nP) \qquad (2.4)$$

Equation (2.2) can now be expressed in terms of the species present.

$$\bar{\nu}_H = \frac{(HP) + 2(H_2P) + 3(H_3P) + \ldots + n(H_nP)}{(P) + (HP) + (H_2P) + \ldots + (H_nP)} \qquad (2.5)$$

Substituting the appropriate quantities from equations (2.1) into (2.5) yields,

$$\bar{\nu}_H = \frac{K_1(H) + 2K_1K_2(H)^2 + \ldots + nK_1K_2\cdots K_n(H)^n}{1 + K_1(H) + K_1K_2(H)^2 + \ldots + K_1K_2\cdots K_n(H)^n} \qquad (2.6)$$

Equation (2.6) may be generalized as

$$\bar{\nu}_H = \frac{\sum_{i=1}^{n} i\left\{\prod_{i=1}^{n} K_i\right\}(H)^i}{1 + \sum_{i=1}^{n}\left\{\prod_{i=1}^{n} K_i\right\}(H)^i} \qquad (2.7)$$

If the n combining sites of the macromolecule P are assumed to be identical and non-interacting, the n conventional equilibrium expressions (equation 2.1) can all be related

to a single equilibrium constant, K_o. According to the Binominal Distribution, the probability (k_i) of precisely i protonations (successes) of n available sites (trials) can be represented as

$$k_i = \binom{n}{i} K_o^i \tag{2.8}$$

where $\binom{n}{i}$ is the i^{th} binominal coefficient obtained from the series expansion of

$$(1+K_o)^n = 1 + nK_o + \frac{n(n-1)}{2!}K_o^2 + \dots \tag{2.9}$$

or, in general,

$$(1+K_o)^n = 1 + \sum_{i=1}^{n} \frac{n!}{i!(n-i)!} K_o^i \tag{2.10}$$

This approach indicates that the macromolecular form (HP) is a composite of n, identical (H^*P) species, each of which may be described by the equilibria

$$K_o = \frac{(H^*P)}{(H)(P)} \; ; \; (P) \text{ is unique.} \tag{2.11}$$

Thus,

$$K_1 = n K_o . \tag{2.12}$$

Similarily, (H_2P) is a composite of n(n-1)/2! identical forms of the species (H_2^*P), and

$$K_1 K_2 = \frac{(H_2P)}{(H)(P)} = \frac{n(n-1)}{2!} K_o^2 . \tag{2.13}$$

In general, then

$$\prod_{i=1}^{n} K_i = \frac{n!}{i!(n-i)!} K_o^i \quad . \tag{2.14}$$

Substituting equation (2.14) into (2.7) yields

$$\bar{\nu}_H = \frac{\sum_{i=1}^{n} i \left\{ \frac{n!}{i!(n-i)!} \right\} (K_o H)^i}{1 + \sum_{i=1}^{n} \left\{ \frac{n!}{i!(n-i)!} \right\} (K_o H)^i} \tag{2.15}$$

Equation (2.15) may be simplified since the denominator is of the same form as the right-hand side of equation (2.10) and hence, may be replaced by the quantity $(1+ K_o H)^n$. The numerator, because of its similarity to the denominator, may also be reduced since

$$\sum_{i=1}^{n} i \left\{ \frac{n!}{i!(n-i)!} \right\} (K_o H)^i = K_o H (1+K_o H)^{n-1} = K_o H \frac{d(1+K_o H)^n}{d(K_o H)} \tag{2.16}$$

Equation (2.15) then becomes

$$\bar{\nu}_H = \frac{K_o H (1+K_o H)^{n-1}}{(1+K_o H)^n} = \frac{n K_o H}{1 + K_o H} \tag{2.17}$$

where $\bar{\nu}_H$ is the average number of protons bound,

n is the total number of combining sites,

K_o is the proton binding constant, and

H is the unbound proton concentration.

The above expression, however, needs modification since the assumption of non-interacting sites is usually invalid. To compensate for this interaction, the free energy function

is defined as

$$\Delta F^{\circ} = \Delta F^{\circ}_{Int} + RT\phi(\bar{\nu}_H) \tag{2.18}$$

or,

$$K_o = K_{Int} e^{-\phi(\bar{\nu}_H)} \tag{2.19}$$

Equation (2.18) indicates a separation of the work of ionization of a particular acidic site into two parts: ΔF°_{Int} measures the work of separation of a proton from the group and, $\phi(\bar{\nu}_H)$ measures the work of removing the proton to infinity against the electrostatic forces of all other charged groups on the polyacid. Tanford (1967) has shown that by approximating the average free energy per molecule in a solution by the free energy of the average molecule (characterized by the average charge $\bar{Z}$),

$$\phi(\bar{\nu}_H) = 2\omega z_i \bar{Z} \tag{2.20}$$

where ω is the electrostatic work function, z_i is the proton charge, and $\bar{Z}$ is the average polymeric charge.

Equation (2.19) may now be written as

$$K_o = K_{Int} e^{-2\omega z_i \bar{Z}} \tag{2.21}$$

and substituted into equation (2.17), which yields after rearrangement,

$$pK_o = pH - \log \frac{\bar{\nu}_H}{n - \bar{\nu}_H} = (pK_{Int})_{Diss} - 0.868\omega\bar{Z} \tag{2.22}$$

where $(pK_{Int})_{Diss}$ is the intrinsic acid dissociation constant of the group in question, i.e. the dissociation constant in

the absence of all electrostatic interactions.

Hence, a plot of pH + $\log \frac{\bar{\nu}_H}{n-\bar{\nu}_H}$ versus $\bar{Z}$ yields upon extrapolation to zero polymeric charge, a value for the intrinsic acid dissociation constant of the SNO material in question. However, as will be shown in Section 3, the electostatic correction term (ω) in equation (2.22) must be accurately known in order that intrinsic formation constants may be calculated for SNO-metal-ion associations. The lack of accurate molecular weight values for the natural organic substances studied introduces large errors into the calculation of $\bar{Z}$ which would make later calculations valueless. However, because the acid-insoluble nature of humic acids requires $-\bar{Z} \rangle 0$; the polymeric charge can be represented as $(-\alpha n)$ assuming no binding by counterions where n is the total number of dissociatable groups, and α is the fraction dissociated. Conversely, the number of bound protons ($\bar{\nu}_H$) is equal to $n(1-\alpha)$.

Equation (2.22) may now be written as

$$pK_o = pH - \log \frac{\alpha}{1-\alpha} = (pK_{Int})_{Diss} + 0.868\,\omega n\alpha \,. \qquad (2.23)$$

The experimental procedures whereby the variou parameters of equation (2.23) can be determined will now be discussed.

Experimental

Poly-L-aspartic acid (M.W. 27,000) aquired from Sigma

Chemical Corporation was used to test the experimental technique. The humic acid was obtained from Aldrich Chemical Corporation and the natural organic materials were isolated from both Smith Lake water, located on the University of Alaska campus, and from surface water collected by the R/V Acona from a station 25 nms south of Seward, Alaska in the Gulf of Alaska. The isolation was accomplished by reverse osmometry utalizing Diaflow® ultrafiltration filters UM-10 (M.W. cutoff 16,000) and PM-30 (M.W. cutoff 45,000) for the lake and seawater organics, respectively. The natural organic materials were obtained in the salt-free form by repetative acidification and dialysis of these extracts followed by evaporation under vacuum to dryness.

Previous studies (Gurd and Goodman, 1952) of the acid dissociation of macromolecules required the preparation of a series of solutions (solution-series technique) containing various amounts by weight of protein, acid or base, and sufficient neutral salt to maintain a constant ionic strength. The equivalents of proton either bound to or removed from the macromolecule at any pH is equal to the difference between the equivalents of standard acid or base added to the polymer solution and the equivalents of proton which remain at equilibrium. The residual, or equilibrium, hydrogen ion concentration is calculated per sample from the experimental pH obtained from a cell employing a liquid junction.

To ensure the accuracy of these determinations, various assumptions regarding electrochemistry must be made (Tanford, 1955). The most important of these are that junction-potential effects are minimized by the presence of a high, constant, ionic strength (μ=0.1) and that the computation of $\gamma^{\pm}$ is independent of the organic concentration.

Difficulties in isolating from natural water systems the quantities of dissolved organic materials required by the solution-series technique prohibited its use. Therefore, a back-titration procedure was adopted in which a weighed quantity of a natrual orgenic material was dissolved in an excess of CO_2 - free KOH (resulting pH 12.0) and equilibrated at 25°C. The ionic strength of this solution was adjusted to 0.15 with KCl to reduce errors in the junction-potential term caused by ionic strength variations occuring during the course of the titration. After each titrant (0.1 N HCl) addition, a ten-minute period of stirring was observed to allow equilibration before measurement. The pH meter employed was a Coleman Model 37A utilizing shielded electrodes. This was standardized against both 0.5 m phthlate and 0.01 m Borax buffers during the course of the titration of ensure accuracy in the important regions of pH. The hydrogen-ion concentrations were obtained from pH values corrected for both volume and ionic-strength variations, (Debey-Huckel Theory).

Results and Discussion

To estimate the magnitude of the error between the numerous-solution method and the titration procedure, the $(pK_{Int})_{Diss}$ of poly-L-aspartic acid, a synthetic polymer, was determined by both techniques. As seen in Table 2, these values not only agree within experimental error but also agree with those values expected for β-carboxylic acids.

Curve (a) of Figure 1 is representative of the results obtained for natural organic materials by the back-titration procedure and indicates protonation of the molecules in the pH regions 3-7 and 8.5-11.0. It was assumed that these regions reflected the protonation of the carboxylic acid and phenolic groups known to be present on these molecules and that the members of each group were identical. The $(pK_{Int})_{Diss}$ was determined for each group type as follows: The inflection points of both curves (a) and (b) of Figure 1 were obtained by a graphical representation of Δ pH/ Δ vol. versus volume. Because the same quantity of KOH was present in each sample, the difference between these points yields a value for the total equilvalents of dissociated carboxylic-acid group per sample, $(H)_T$. The equivalents of proton bound by the organic material per sample, $(H)_{pH}$, at any point on the titration curve, (a) of Figure 1, can be calculated from the equivalents of standard acid added after the inflection point; and the equivalents of proton remaining in solution. The

TABLE 2

β -Carboxyl Compound	$(pK_{Int})_{Diss}$
Poly-L-Aspartic Acid (M.W. 27,000)	
Numerous Solution Technique	4.51
Titration Technique	4.52
N-Carbomoyl-β-Alanine	4.49
Succinic Acid	4.44
N-Acetyl-β-Alanine	4.51

A Comparison Between Observed and Literature Values for the Intrinsic Acid Dissociation Constant of Poly-L-Aspartic Acid

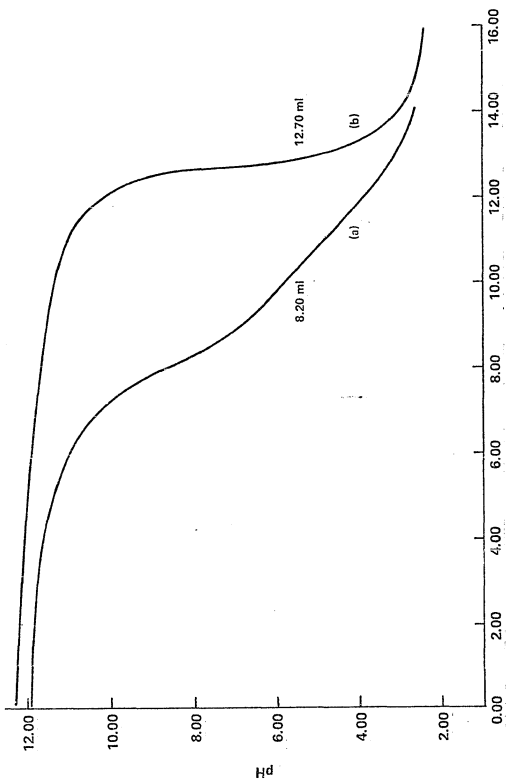
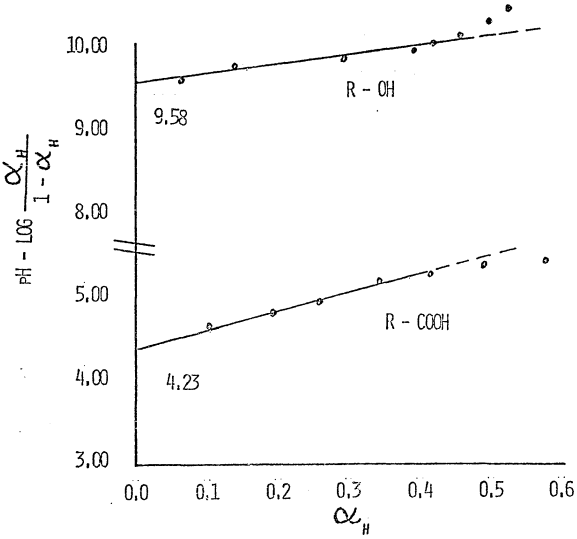


FIGURE 1

latter quantities are calculated from the experimental pH values with required corrections for ionic strength and volume variations. Because $\left[1 - (H)_{pH} / (H)_{T}\right]$ is equal to α_H, the fraction of sites dissociated at a specific pH, a graphical solution to equation (2.21), Figures 2 and 3, may be obtained utilizing data such as that presented in Table 3. A similar arguement exists for the $(pK_{Int})_{Diss}$ calculation of the phenolic groups.

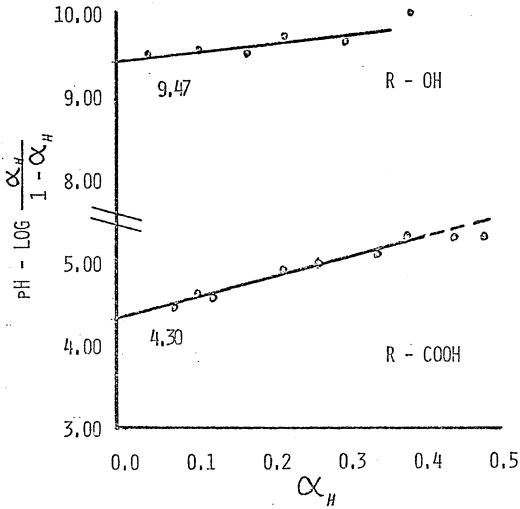
$$pH - \log \frac{\alpha_H}{1 - \alpha_H} = (pK_{Int})_{Diss} + 0.868 w n \alpha_H \qquad (2.21)$$

The constants obtained in this manner for the various humic compounds studied are listed in Table 4 in addition to the values expected for small molecules. The constants obtained for the seawater extract are subject to a large error since once having isolated the required amount of material for a titration (0.09mg), it was impossible to redissolve it in an excess of KOH with any degree of success. On the basis of the assumption of identical groups the high acidity of these materials might be explained in terms of group-unsaturated system-interactions although this is speculative because of the lack of knowledge concerning the structure of the humic substances. Nevertheless, as will be shown in Section 3, the quantities determined by this method are of great importance in the determination of the intrinsic constants of formation for metal-humic associations.



DATA OF TABLE 3 PLOTTED BY EQUATION (2.21) TO DETERMINE THE INTRINSIC DISSOCIATION CONSTANTS OF SMITH LAKE ORGANIC MATERIAL

FIGURE 2



THE INTRINSIC ACID DISSOCIATION CONSTANTS OF ALDRICH HUMIC ACID

FIGURE 3

TABLE 3

Carboxylic Acid Groups

pH	α_H	$1-\alpha_H$	pH - log $\frac{\alpha_H}{1-\alpha_H}$
6.016	0.671	0.329	5.706
5.525	0.529	0.471	5.473
5.030	0.425	0.575	5.160
4.807	0.354	0.646	5.066
4.390	0.264	0.736	4.835
4.009	0.189	0.811	4.641
3.595	0.109	0.891	4.507

Phenolic Groups

pH	α_H	$1-\alpha_H$	pH - log $\frac{\alpha_H}{1-\alpha_H}$
10.036	0.479	0.512	10.072
9.907	0.444	0.556	10.003
9.814	0.416	0.584	9.960
9.494	0.294	0.706	9.873
9.000	0.149	0.851	9.756
8.623	0.088	0.912	9.639

Representative Data Employed in the Calculation of the Intrinsic Dissociation Constants of Smith Lake Organic Material

TABLE 4

Compound	pK^{-COOH}_{Int}	pK^{-OH}_{Int}
Aldrich Humic Acid	4.30	9.47
Smith Lake Organic	4.23	9.61
Seawater Organic	3.95	9.85
Phenol	-	9.78
β -Carboxylic Acids	4.50	-

Intrinsic Acid Dissociation Constants

Determined in this Study

Deviations from linearity such as that observed in Figure 2 could be ascribed to a breakdown in the theory resulting from nonrandom protonation of groups at large α_H values. However, it appears more likely that this deviation is caused by a combination of experimental error and the presence of other carboxylic-acid group-types on the molecules. A detailed analysis of humic titration curves is needed to resolve this dilemma.

Assuming that the values obtained from the slope of the linear portion of curves such as Figure 2 are independent of α_H, equation (2.21) may be employed to estimate the percent of like-groups ionized as a function of pH, Figure 4. Such curves could be of great importance in the estimation of the cation-exchange capacities of humic materials under natural pH conditions.

The apparent equivalent weights, expressed in grams of organic per equivalent of COOH group, were found to be 293 for the seawater organic, 263 for Aldrich humic acid and 195 for the Smith Lake organic material. Barsdate (1967) determined the latter value to be between 200-210 by a similar method which is in good agreement with this work.

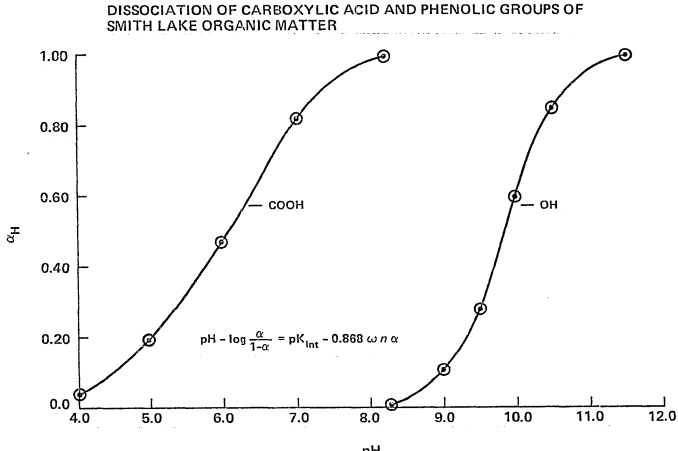


FIGURE 4

SECTION 3

INTERACTIONS BETWEEN SOLUBLE, NATURAL ORGANIC MATERIALS AND METAL-IONS

Having developed, in Section 2, a methology by which the population of sites capable of complex formation can be determined for any SNO ligand as a function of pH, it is now necessary to consider the influence of metal ions on such systems.

General Equations

For the case of two species (H and M) competing for a given site on a macromolecule, three forms of the site are possible: H is present, M is present, or the site is free. If α_H, α_m and $1-(\alpha_H+\alpha_m)$ represent the fraction of all sites in these forms, then substitution into equation (2.17) yields,

$$\frac{\alpha_H}{1-(\alpha_H+\alpha_m)} = K_o^H(H) \text{ and } \frac{\alpha_m}{1-(\alpha_H+\alpha_m)} = K_o^m(M) \qquad (3.1)$$

Combining the above equations and rearranging yields,

$$\frac{\alpha_m}{1-\alpha_m} = \frac{K_o^m(M)}{1+K_o^H(H)} \quad . \qquad (3.2)$$

If only a single type of site is involved in the competitive reactions between hydrogen ions and metal ions and, if the only interactions between sites are electrostatic in nature, then by the application of equation (2.18), the association constants for both H and M can be described as

follows: $$k_o^M = (K_{Int})_M e^{-2\omega z_m \bar{Z}} \quad (3.3)$$

and $$k_o^H = e^{-2\omega \bar{Z}} / (K_{Int})_{Diss} \quad (3.4)$$

where $(K_{Int})_{Diss}$ is the intrinsic acid dissociation constant of the binding groups on the macromolecule, $(K_{Int})_M$ is the intrinsic metal-ion formation constant quantifying the reaction between a given transition metal and the available SNO binding sites, z_M is the charge of the metal ion, ω is the electrostatic interaction factor and, $\bar{Z}$ is the polymeric charge at specific pH values.

Substitution of equations (3.3) and (3.4) into (3.2) yields equation (3.5), an expression by which $(K_{Int})_M$ can be determined.

$$\frac{\alpha_m}{1-\alpha_m} = \frac{(K_{Int})_m e^{-2\omega z_m \bar{Z}(M)}}{1 + \frac{e^{-2\omega \bar{Z}(H)}}{(K_{Int})_{Diss}}} \quad (3.5)$$

Although equation (3.5) has been used extensively in the study of proteins (Tanford and Hauenstein, 1956; Gurd and Goodman, 1952), it is unsuitable in its present form for a study of SNO complexation since the polymeric charge ($\bar{Z}$) can not be accurately evaluated without a knowledge of the average-molecular-weight of the ligand. A further complication arises because the polymeric charge at a given pH must be corrected for the presence of bound metal ions.

This situation can be circumvented by representing the polymeric charge per molecule as

$$\bar{Z} = -\alpha_H n_H + 2(\bar{\nu}_m) \qquad (3.6)$$

where $2(\bar{\nu}_m)$ corrects for the presence of $\bar{\nu}_m$ bound metal ions of +2 valence. The quantity $\bar{\nu}_m$ can also be expressed in terms of the total number of sites occupiable by metal ions per molecule (n_M) multiplied by the fraction of sites occupied at a given pH,

$$\bar{\nu}_m = n_m \alpha_m \qquad (3.7)$$

Substituting (3.7) into (3.6) yields,

$$\bar{Z} = -\alpha_H n_H + 2 n_m \alpha_m \qquad (3.8)$$

Defining γ as the ratio of the total number of sites occupiable by the metal ions per molecule (n_M) divided by the total number of titratable carboxylic-acid groups per molecule (n_H), a dimensionless constant is obtained which may then be determined from the study of any weighed quantity of SNO substances.

$$\gamma = \frac{n_m}{n_H} \qquad (3.9)$$

Substituting (3.9) into (3.8) gives

$$\bar{Z} = n_H\left[-\alpha_H + 2\gamma\alpha_m\right] \qquad (3.10)$$

which, when combined with equation (3.5) yields the practical expression,

$$\frac{\alpha_m}{1-\alpha_m} = \frac{(K_{Int})_m e^{4\omega n_H\left[\alpha_H - 2\gamma\alpha_m\right]}\cdot(m)}{1 - \dfrac{(H)}{(K_{Int})_{Diss} e^{-2\omega n_H\left[\alpha_H - 2\gamma\alpha_m\right]}}} \qquad (3.11)$$

where the variables retain their previously defined identity. Since ω n_H, α_H, $(K_{Int})_{Diss}$ and n_H (per gram of humic) have previously been determined for the various natural organic materials studied in Section 2, only the number of moles of metal-ion bound per gram of humic substance need be determined in order to calculate the required intrinsic formation constants by equation (3.11).

Experimentally, the metal-ion binding of humic materials was studied by two independent techniques: dialysis equilibration and Electron Spin Resonance (ESR) spectroscopy. These techniques will be discussed below.

Dialysis Equilibration

Chemicals and Equipment. The isolation and purification of the organic materials used in this study, i.e. Smith Lake organic and Aldrich humic acid, have been discussed in Section 2. Stock solutions were prepared for each organic by dissolving a quantity of material in excess KOH, followed by neutralization and filtration employing a 0.45 micron Millipore® membrane. The filtrates were diluted to the desired volume after adjusting the ionic strength to 0.1 with KNO_3. Three 50 ml aliquots of each organic solution were repeatedly acidified and dialyzed, then evaporated to dryness to determine the grams per liter of the humic material present.

Six dialysis chambers of 50 ml total volume were constructed from 1 inch I.D. Pyrex® glass pipe. Two inch squares

of dialysis tubing (single thickness) separated the compartments and were firmly secured by aluminum flanges. These tubes were attached when filled to a mechanical shaker for a 24 hour period to ensure equilibration.

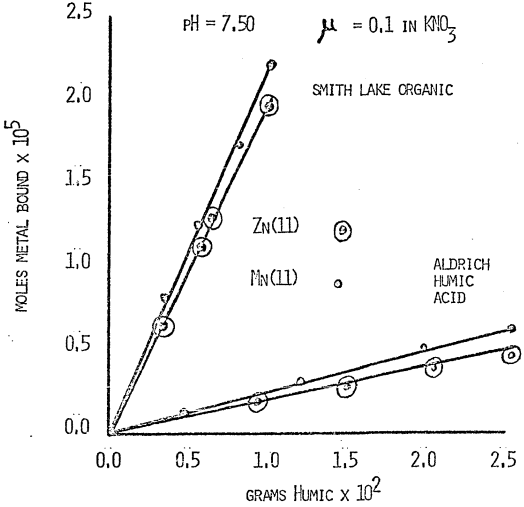
Stock solutions of Mn (11), Zn (11) and Cu (11) were prepared from nitrate salts to be 0.01 M/L and were standardized by EDTA titrations. Solutions utilized in these experiments were dilutions of the above and contained approximately 10^{-4} M/L at $\mu = 0.1$ in KNO_3. A Perkin-Elmer Atomic Absorption Model 303 Spectrophotometer was employed to measure both the zinc (11) and copper (11) equilibrium concentrations on both sides of the membrane. Mass balances were obtained for Mn (11) as the permanganate, utilizing a Beckman D.U. Spectrophotometer. When required, interfering organic matter was first destroyed by persulfate oxidation.

Procedures and Discussion. Twenty-five mls of a standard solution of the appropriate metal-ion was introduced to one side of the membrane of each dialysis cell. Various amounts of the respective organic solution and sufficient 0.1 M/L KNO_3 solution to give 25 mls was introduced to the other side. The high ionic strength of the system was necessary to suppress the Donnan effect and to ensure the equality of the activity coefficients of the free metal ions on both sides.

The organic concentration was varied over the range 0 to 30 mg per sample for the Aldrich humic acid and from 0 to 10 mg per sample for the Smith Lake organic. The hydrogen-ion concentrations of these systems were adjusted to 7.5 with either HCl or KOH to insure the maximum dissociation of humic binding groups (COOH) while minimizing interferences caused by metal-hydroxy complex formation. It was found that at the humic concentrations employed, metal-ion molarities of 1.0×10^{-3} were sufficient to saturate the organic bonding sites. Aliquots were withdrawn from each side of the membrane after equilibration. The moles of metal-ion bound per gram of organic material was calculated from the excess metal found on the organic side of the membrane and the volume of that compartment.

Figure 5 indicates that the amount of metal-ion bound is a linear function of the organic concentration present at equal volumes of solution and constant metal-ion concentration. It is also seen that the number of sites occupied is independent of the metal studied and depends soley on the humic material in question.

The maximum binding capacities of the organic materials studied are determined from the slopes of their respective curves in Figure 5. These values coupled with the apparent equivalent weights of the humic substances (280 g/equiv.- Aldrich humic acid; 195 g/equiv.- Smith Lake organic material)

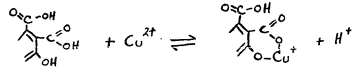


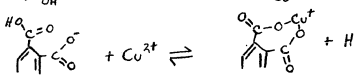
MOLES OF METAL BOUND BY HUMIC MATERIALS AS A FUNCTION OF ORGANIC CONCENTRATION

FIGURE 5

allow the calculation of the fraction of titratable sites occupied by metal ions, γ in equation (3.9). These values were determined to be 0.06 and 0.39 for Aldrich humic acid and Smith Lake organic material, respectively. Although the value obtained for the Smith Lake material is in accord with the 0.36 reported by Barsdate and Fu (1971) for the same substance, the magnitude of γ for Aldrich humic acid appears unrealistic. However, this material was chosen for its accessibility; information concerning both its origin and isolation are lacking.

If Gamble, et al. (1970) are correct that reactions between humic and fulvic acids and divalent metal ions proceed essentially via the following two mechanisms,

a) 

b) 

the value of 39% carboxylic-acid site occupation for Smith Lake organic material would indicate that very few of the possible binding sites are suitable for metal-ion interaction. This situation is even more drastic for Aldrich humic acid.

To determine the binding capacity of humic materials as a function of pH, experiments were performed as describ-

ZINC-SMITH LAKE ORGANIC

pH	α_{Zn}	α_H	$(Zn^{+2})_{free}$	pK_{Int}
4.063	0.150	0.170	2.87×10^{-4} M/L	3.50
4.356	0.138	0.220	2.89×10^{-4} "	3.35
5.162	0.400	0.375	1.92×10^{-4} "	3.27
5.780	0.724	0.496	1.20×10^{-4} "	2.71
				3.21 average

$\omega_{n_H} = 3.13$

$\gamma = 0.39$

$(K_{Int})_{Diss} = 5.87 \times 10^{-5}$

COPPER-ALDRICH HUMIC ACID

pH	α_{Cu}	α_H	$(Cu^{+2})_{free}$	pK_{Int}
3.946	0.063	0.160	5.74×10^{-5} M/L	3.18
4.063	0.157	0.185	5.46×10^{-5} "	3.53
4.902	0.294	0.380	5.26×10^{-5} "	3.20
5.048	0.458	0.410	4.60×10^{-5} "	3.12
				3.28 average

$\omega_{n_H} = 2.50$

$\gamma = 0.09$

$(K_{Int})_{Diss} = 5.05 \times 10^{-5}$

Sample Data Employed for the Calculation

Intrinsic Formation Constants

TABLE 5

ed above with the exception that both the humic and metal-ion concentrations were maintained constant such that saturation of the binding sites was insured under optimum conditions. The quantities of Aldrich humic acid, Smith Lake material, and metal-ion employed per 25 ml sample were 8.9 mg, 4.0 mg, and 5.0 x 10^{-3} mmoles, respectively. The pH of each solution was adjusted to specific values in the range 4.60 to 8.00. The results obtained from the analysis of the equilibrated systems are presented in Figure 6, where α_M is the fraction of the total available metal-binding sites occupied.

Having determined both α_M as a function of pH and the concentration of free metal ion in a given sample, these values can be combined with those previously determined parameters α_H, ωn_H, $(K_{Int})_{Diss}$, and γ, and the intrinsic formation constants calculated by means of equation (3.11), Table 5, for the associations studied.

The constants listed in Table 6 do not substantiate the hypothesis that humic materials are strong chelators. From equation (3.3) it is seen that, although the intrinsic formation constant is invariant, the apparent formation constant (K_o^M) clearly depends upon α_H. It is evident

$$K_o^M = (K_{Int})_M \, e^{+2\omega z_m n_H \alpha_H} \qquad (3.3)$$

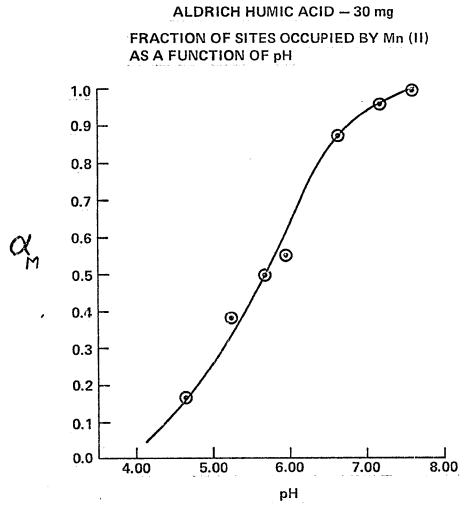


FIGURE 6

TABLE 6

	$(pK_{Int})_{Metal}$		
Organic	Mn (II)	Zn (II)	Cu (II)
Smith Lake	2.50	3.37	3.68
Aldrich Humic	2.24	3.29	3.19

The Intrinsic Formation Constants Determined for Metal-Humic Systems by Dialysis Equilibration

then, that the observed binding power of humic materials is caused by the large population of bonding sites available to a metal-ion under natural pH conditions.

Electron Spin Resonance (ESR)

Cohn and Townsend (1954) first utilized an ESR to determine the formation constants of a few well-known organic-Mn(11) associations. For reasons which are discussed in Section 4, the $Mn(H_2O)_6^{+2}$ species exhibits a sharp paramagnetic absorption in solution. In the formation of a complex, however, the replacemant of water molecules by other ligands broadens the paramagnetic absorption of the manganous ion to a point where is is not experimentally observable. Thus, the concentration of the uncomplexed Mn(11) in a given system can be determined from the intensity of the narrow resonance.

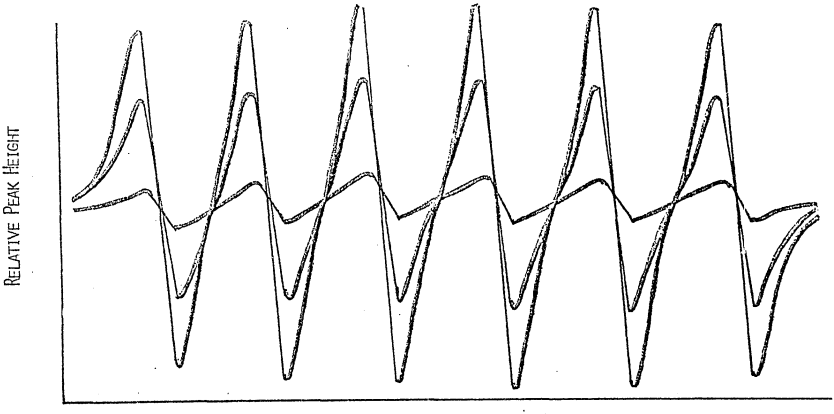
Because Cohn and Townsend (1954) have shown that the height of the derivative curve is a linear function of the Mn(11) concentration in the range 10^{-3} to 10^{-5} M/L and, because subsequent work (Appendix 1) has proven this technique to be applicable to both simple and polymeric ligands; an ESR investigation of Mn(11)-humic associations was justified.

Experimental. It was first necessary to ascertain whether the ESR technique was applicable to other metal ions. The metals studied were copper (11), iron (11), nickel (11), cobalt (11) and manganese (11). It was found that both copper and iron were unsuitable for this method since the

width of the hyperfine spectrum broadened greatly under complexation. Although the height of the derivative peak was found to be a linear function of concentration, the study of nickel and cobalt was also discontinued since the concentrations required to obtain good spectra were on the order of 0.1 to 0.01 M/L. Manganese (11) proved to be quite sensitive, detectable at 10^{-5} M/L, and the study was continued with this species assuming that the results could be extrapolated to other transition metal ions.

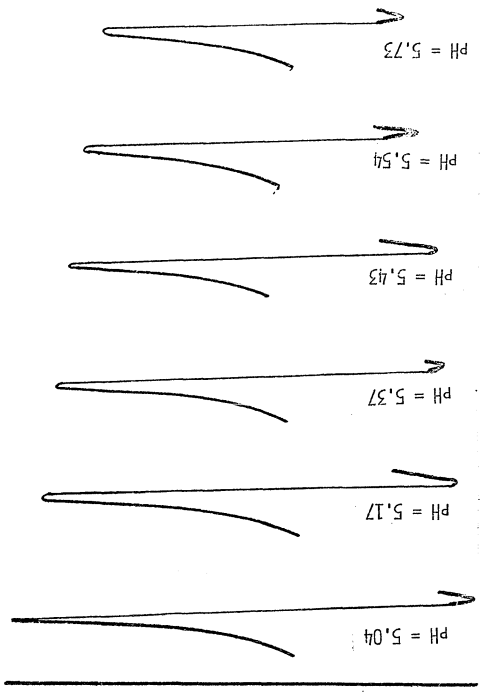
Solutions were prepared which contained, upon dilution to 50.0 mls, either 17.8 mg of Aldrich humic acid (AHA) or 6.0 mg of Smith Lake organic material (SLO) and 2.0 x 10^{-3} mmoles of Mn(11) at an ionic strength of 0.1 in KNO_3. The pH of each was adjusted with either HCl or KOH to values in the range 4.00 to 8.00 and the samples equilibrated at 25° for twenty-four hours. Three-ml aliquots were then withdrawn and injected via syringe into an ESR liquid cell and the Mn(11) hyperfine spectra recorded at the following instrument settings; field-3400 Gauss, frequency-9.475, and field range-$\pm$500 Gauss. The height of the derivative curve of each sample was compared to a standard curve of Mn(11) concentration verus peak height to determine the moles of cation bound.

Results and Discussion. Figures 7 and 8 are representative of the results obtained. The fraction of titratable groups (COOH) occupied by manganous ions, δ defined



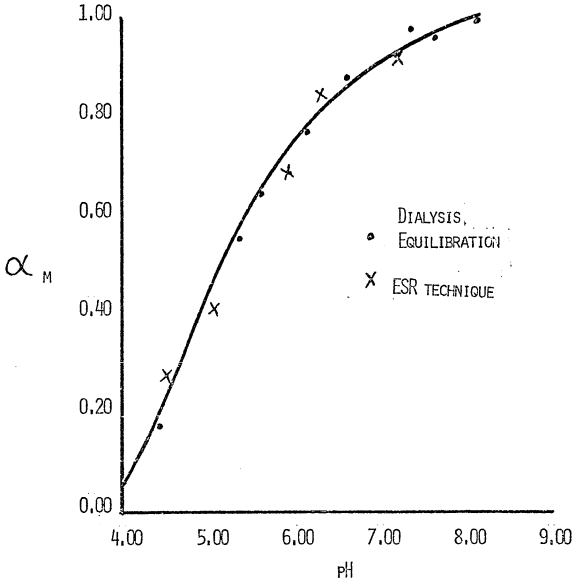
DECREASE IN THE HYPERFINE SPECTRUM OF MN(II) AS A RESULT OF BINDING BY HUMIC MATERIALS

FIGURE 7



DECREASE IN THE HYPERFINE SPECTRUM OF MN(II) UPON COMPLEXATION BY ALDRICH HUMIC ACID AS A FUNCTION OF PH

FIGURE 8



A COMPARISON OF α_M VALUES FPR MN(11)-ALDRICH HUMIC ACID BY ELECTRON SPIN RESONANCE AND DIALYSIS STUDIES

FIGURE 9

in Section 3, was calculated for each organic from the number of moles cation bound divided by the product of the sample concentration expressed in grams and the apparent equivalent weight as determined in Section 2. The values obtained, AHA-0.09 and SLO-0.46, are in good agreement with those determined by the dialysis studies.

The ESR technique also allows the calculation of α_m, the fraction of total metal-ion binding-sites occupied at specific pH values, and, thus, provides a check on the intrinsic formation constants determined for the manganese-humic systems by dialysis equilibration. Figure 9 shows that the calculated values of α_m are in excellent agreement between the two techniques.

SECTION 4

THE MECHANISM OF METAL-HUMIC ASSOCIATIONS

The practice of describing metal-humic interactions as coagulation, peptization, surface adsorption, and chelation reactions (Mortensen, 1963) should be discontinued because of redundancy. As will be shown below, each of these processes can be directly related to the Law of Mass Action; hence they are experimentally indistinguishable.

Coagulation and peptization are, respectively, manifestations of the formation and rupture of electrostatic interactions between charged particles. Since the surface charge of a particle is a direct function of the amount of polar additive bound, surface adsorption reactions are the controling influence.

The adsorption equation of Langmuir is based upon a theoritical consideration of the process of adsorption. This equation may be written

$$\frac{X}{M} = \frac{kC}{1+kC} \qquad (4.1)$$

where X is equal to the amount of material in grams adsorbed,

M is the weight in grams of adsorbant,

C is the concentration of free adsorbing species,

and, k is a constant.

However, the Langmiur equation is actually a description of chemical equilibrium, which may be seen from the following arguement.

If R is the part of an ion-exchange resin which combines with a cation according to the reaction

$$R + M \rightleftharpoons RM \tag{4.2}$$

the constant for the association is

$$k = \frac{(MR)}{(M)(R)} \tag{4.3}$$

Because the total active resin concentration is

$$(R_t) = (RM) + (R) \tag{4.4}$$

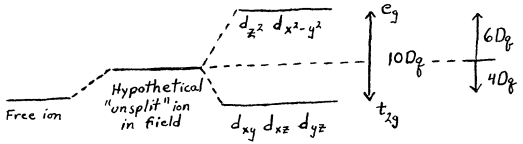
the reaction may be described by equation (4.5).

$$\frac{(RM)}{(R_t)} = \frac{k(M)}{1 + k(M)} \tag{4.5}$$

Equation (4.5) is also identical to that derived in Section 2 (equation 2.17) which describes the complexation, either chelation or ion-pair, of a metal ion by a soluble, organic ligand. It is apparent then, that the present mechanistic interpetations of metal-humic associations are valueless.

Schnitzer and Hansen (1970) have stated that the metal-humic formation constants determined by the ion-exchange technique obey the "natural order" of stability, $Mn^{+2} < Fe^{+2} < Co^{+2} < Ni^{+2} < Cu^{+2} > Zn^{+2}$. Although only three metals were studied, a similar result was observed for this work.

This phenomena is readily explained by crystal field theory. In an octahedral complex, two of the d orbitals, d_{z^2} and $d_{x^2-y^2}$, are directed toward the ligands. The replusion caused by the ligands raises the energy of these orbitals(e_g) more than that of the other three orbitals, d_{xy}, d_{xz} and d_{yz} (t_{2g}), which are directed at 45° to the axes. The stronger



the field due to the ligands, the greater the splitting of the energy levels($10D_q$). If all five orbitals are equally occupied, d^0, d^5(high spin) and d^{10}, there is no change in the energy of the system due to splitting. For all other configurations, however, the splitting lowers the total energy of the system. The decrease in energy caused by splitting is the crystal field stabilization energy(CFSE).

In a weak field, the splitting is small and the addition of electrons to the empty orbitals follows Hund's rule because the possible gain in CFSE is not sufficient to provide the required electron pairing energy. This is referred to as a high spin case because there is the maximum number of unpaired electrons.

Conversely, in a strong field situation, the gain in CFSE

is sufficient to cause electron pairing in the t_{2g} orbitals before population of the e_g orbitals occurs. Table 7 shows the CFSEs expected for the first transition series for both high and low spin octahedral complexes.

It has been found by experimental study of the spectra of numerous complexes that ligands do not behave as point charges but, depending on their structure, possess varying capacities to cause d-orbital splitting. Thus, common ligands may be arranged according to their splitting abilities; the spectrochemical series.

$$I^- < Br^- < Cl^- < OH^- < R\text{-}COO^- < H_2O < pyridine < CN^-$$

In the formation of a complex, water molecules are displaced by ligands. If the metal-ion concerned is subject to crystal field stabilization, this stabilization will be greater in the complex than that in the aquo-ion, if the ligand is further along in the spectrochemical series than is H_2O. However, d^0, d^5(high spin) and d^{10} configurations experience no CFSE in either the hexaquo-ion or in the complex so that complexation cannot cause any increased stabilization. Similarly, of two ions, both of which experience crystal field stabilization, the one which experiences the greater amount from both the ligand and from H_2O will also experience the larger increase on replacement of H_2O by the ligand. Thus, the order of the ions in the "natural" stability series follows their order in regard to CFSEs (Table 7) with the ex-

Configuration	Examples	Strong Field t_{2g}	Strong Field e_g	Strong Field No. of unpaired e⁻	Strong Field CFSE(-Dq)	Weak Field t_{2g}	Weak Field e_g	Weak Field No. of unpaired e⁻	Weak Field CFSE(-Dq)
d^0	Ca(11)	0	0	0	0	0	0	0	0
d^1	Ti(111)	1	0	1	4	1	0	1	4
d^2	V(111)	2	0	2	8	2	0	2	8
d^3	Cr(111)	3	0	3	12	3	0	3	12
d^4	Cr(11)	4	0	2	16	3	1	4	6
d^5	Mn(11)	5	0	1	20	3	2	5	0
d^6	Fe(11)	6	0	0	24	4	2	4	4
d^7	Co(11)	6	1	1	18	5	2	3	8
d^8	Ni(11)	6	2	2	12	6	2	2	12
d^9	Cu(11)	6	3	1	6	6	3	1	6
d^{10}	Zn(11)	6	4	0	0	6	4	0	0

Crystal Field Stabilization Energy of Octahedral Complexes

TABLE 7

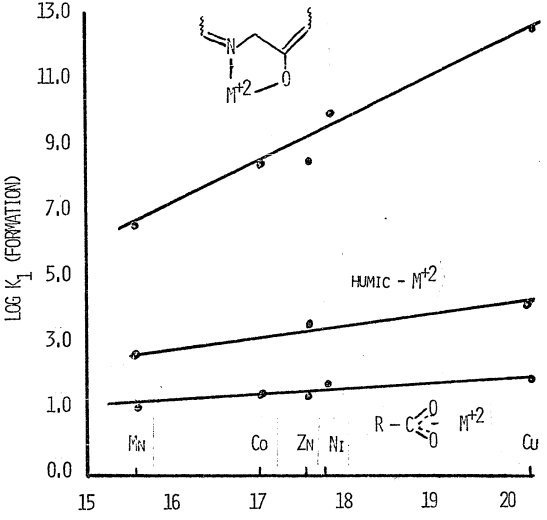
(Douglas and Mc Daniel, 1965)

ception of Cu(11) relative to Ni(11). Thsi discrepancy has yet to be resolved.

Because the intrinsic metal-ion formation constants determined in Section 3 are, by definition, independent of electrostatic effects, they may be compared to similar thermodynamic constants for simple ion-pair and chelate systems. In Figure 10, the ionization potentials of the first transition series are plotted against the first formation constant for two model ligands. The ionization potential of a metal is related to the tendency towards complex formation since it measures the attraction of the cation for electrons.

The acetate ion has been employed as an example of an ion-pair system and 8-hydroxyquinoline as a representative chelate. The values obtained for Aldrich humic acid have also been included. Although by no means conclusive, Figure 10 indicates a greater stability for humic associations than would be expected for pure electrostatic interactions. To examine the possible covalent contribution to the bond energies of humic complexes, the ESR spectrophotometer was again employed. However, as discussed below, newly-uncovered evidence has generated doubts regarding the reliability of the obtained data.

Cohn and Townsend (1954) attributed the ESR hyperfine spectrum of Mn(11) in solution to the admixture of two electronic configurations, one of which contains the 4s orbital



PLOT OF THE SECOND IONIZATION POTENTIAL OF THE METAL AGAINST THE FIRST FORMATION CONSTANT OF SOME COMPLEXES

FIGURE 10

Consequently, in the case of covalent bond formation, electrons donated by another atom in the complex occupy the 4s orbital of the manganous ion inhibiting the mixing of these configurations and resulting in the disapparance of the hyperfine spectrum.

This thesis was reinforced by Guilbault and Meisel (1969) who explored the use of ESR as a precise analytical method for Mn(11) determinations in solutions of diverse substances. Their results indicate that ligands such as acetate, Cl^- and Br^-, which are known to form ion-pair associations with Mn(11), have negligable effects on the manganous hyperfine spectrum except at high concentrations and, that then, the observed variations could be attributed to physical factors such as changes in solution viscosity and dielectric loss in the sample cavity. In addition, these authors found that chelating agents (EDTA, oxalate and tartrate) greatly reduced the Mn(11) hyperfine spectrum when present in amounts small compared to the manganous ion.

However, evidence is available negateing the bond discrimination ability of the ESR method. Malmstrom, et al. (1958) stated that covalent bond formation cannot be invoked because the hyperfine spectrum disappears completely in Mn(11)-2-phosphoglutaric acid, a complex they have shown to involve purely ionic coordination. Similarly, Mc Garvey (1957) both determined the formation constant of Mn(11)-pyridine by ESR and measured the magnetic susceptibility of the solid complex.

His value of 5.97 Bohr magnetons per manganese agrees closely with the 5.90 obtained by equation (4.6) which assumes that the magnetic moments of the first transition series can be calculated from the spin contribution alone.

$$\mu_m \text{ (Bohr Magnetons)} = \sqrt{n(n+2)} \qquad (4.6)$$

where n is the number of unpaired electrons

Thus, the ground state multiplicity of the metal ion is unaltered by pyridine, a strong field ligand.

Mc Garvey (1957) further stated that the replacement of H_2O by complexing species reduces the electric field symmetry of the $Mn(H_2O)_6^{+2}$ ion. This reduction leads, in turn, to a broadening of the resonance absorption band of the complex to a point where it is not observable under normal operating conditions. Thus, only the free Mn(11) concentration is detected.

Although the available evidence indicates that the disappearance of the Mn(11) hyperfine spectrum is not an acceptable criteria for covalent bond formation, the possibility still exists because the 4d orbital should be low enough in energy in a positive transition metal ion to be used for covalent bond formation (Douglas and Mc Daniel, 1965). Hence, the mechanism of interaction between humic materials and metal ions is still a matter for speculation.

SECTION 5
FINDINGS

1) The approach developed in Section 2 and 3 is the best available interpetation of metal-humic complexation in as much as the derived equations compensate for both the random ionization of, and the electrostatic interactions between, bonding groups.

2) The equations of Section 3 allow the calculation of intrinsic formation constants without the need of average-molecular-weight values for the ligand studied.

3) The experimental procedures by which the intrinsic acid dissociation constants were determined require further refinement to insure the accuracy of the obtained values.

4) The Electron Spin Resonance spectrophotometer has been shown to be a useful technique for the investigation of Mn(11) associations regardless of ligand complexity.

CONCLUSION

The interactions between a metal-ion and a binding site on a humic ligand may be described, on the basis of the determined intrinsic formation constants, as possessing only modest strength. However, the large population of groups capable of complexation on a humic molecule increases the overall effect of the organic concentration to where it is theoretically capable of competeing with other ligands for trace metal ions. The equations developed in this dissertation are, however, unable to predict the extent of this

competition because the absence of the organic concentration (P) in the equilibrium expression inhibits the incorporation of equation (3.11) into the existing thermodynamic models of seawater chemical speciation. In addition, this approach excludes complexes which contain more than one molecule of polymer. These complex types may be of importance in systems which possess high organic and low metal-ion concentrations. Thus, this approach requires further refinement if a comprehensive, equilibrium model for natural water systems is to be realized.

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APPENDIX 1

THE APPLICATION OF ELECTRON SPIN RESONANCE (ESR) TO THE STUDY OF Mn(11) ASSOCIATIONS WITH SIMPLE AND MACROMOLECULAR LIGANDS

The sparseness of the data in the literature concerning the study of manganese (11) complexation reactions by ESR spectroscopy indicated that before humic systems could be studied, simple ligands should first be employed to explore the feasibility of this technique in the study of complexation reactions. If these results proved satisfactory, the study would be expanded to include the polymeric ligands poly-aspartic acid (PAA) and bovine serum albumin (BSA), and finally humic materials.

The theoretical background of the ESR technique was previously discussed in Section 3 as were the results for complexation by natural substances and, hence, will not be considered here.

Experimental

Tiron (1,2-dihydroxybenzene-3,5-disulfonic acid disodium salt), 8-hydroxyquinoline, Na_2HPO_4 and disodium EDTA were obtained from K & K Laboratories, Inc.. Poly-aspartic acid (M.W. 24,000) and bovine serum albumin (M.W. 69,000) were obtained from Sigma Chemical Corporation.

The manganese (11) solutions were prepared from reagent

grade manganese sulfate monohydrate. Sample solutions were prepared to contain 2.00 x 10^{-4} M/L Mn(11) and either 1.0 x 10^{-3} M/L of simple ligand, 7.0 x 10^{-5} M/L BSA, or 4.2 x 10^{-6} M/L PAA at an ionic strength of 0.1 in KNO_3. The hydrogen-ion concentrations were adjusted to specific values in the pH range 2-8.5 by the addition of either KOH or HCl. Hydrogen-ion concentrations were determined with a Coleman Model 37A pH meter utilizing a glass-calomel electrode system. Following equilibration, 1.0 ml of each sample was introduced into an ESR liquid cell and the spectra obtained with a Varian Model E-3 spectrophotometer. The instrument settings were: field- 3400 G, frequency- 9.475 and field range- ± 500 G. The sample spectra were compared to a standard linear curve of peak height versus Mn(11) molarity to obtain the concentration of unbound metal ion.

Results and Discussion

Simple Ligands

The formation constant for the generalized equilibria

$$Mn^{2+} + A^{-} \rightleftharpoons MnA^{+} \qquad \text{(A-1.1)}$$

may be described by the following equation

$$K_f = \frac{(MnA^{+})}{(Mn^{2+})(A^{-})} \qquad \text{(A-1.2)}$$

Since for a 1:1 complex,

$$F_{Mn^{2+}} = (Mn^{2+})_{free} + (Mn\dot{A}) \qquad \text{(A-1.3)}$$

$$F_{HA} = (HA) + (A^{-}) + (MnA^{+}) \qquad \text{(A-1.4)}$$

Equation (A-1.2) may be rearranged yielding

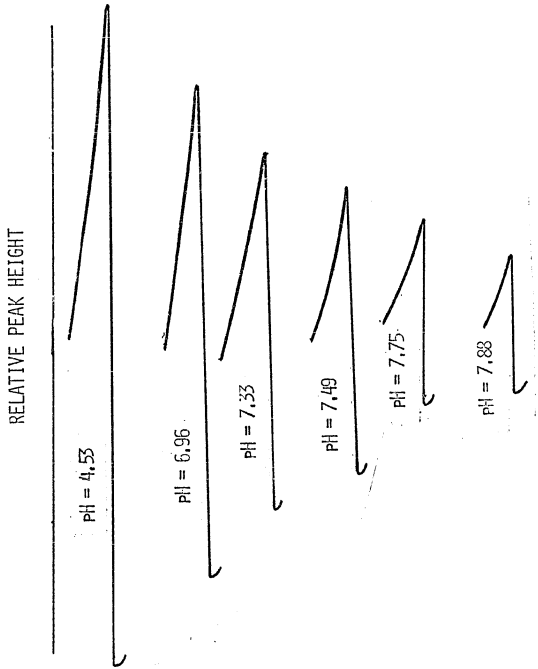
$$K_f = \frac{\left\{F_{Mn^{2+}} - (Mn^{2+})_{free}\right\}\left\{(H)/K_A + 1\right\}}{(Mn^{2+})_{free}\left\{F_{HA} - F_{Mn^{2+}} - (Mn^{2+})_{free}\right\}} \qquad \text{(A-1.5)}$$

Hence, because the acid dissociation constant of the complexing species (K_A) and the formal concentrations of the metal ion and ligand are known, only the pH and the free metal-ion concentration need be determined to calculate the required K_f. Figure 11 is representative of the results obtained and, Table 8 lists the pK_f values for the equilibria studied.

Macromolecular Ligands

The intrinsic formation constants describing the reactions between Mn(11) and the polymeric ligands BSA and PAA were calculated by equation (3.5).

$$\frac{\bar{\nu}_{Mn}}{n - \bar{\nu}_{Mn}} = \frac{(K_{Int})_{Mn}\, e^{-4\omega\bar{Z}} \cdot (Mn^{2+})_{free}}{1 + \dfrac{e^{-2\omega\bar{Z}}(H)}{(K_{Int})_{Diss}}} \qquad (3.5)$$



DECREASE IN THE Mn(II) ESR HYPERFINE SPECTRUM UPON CHELATION BY TIRON (DISODIUM-2, 3-DIHYDROXYBENZENE DISULFONATE) AS A FUNCTION OF pH

FIGURE 11

Ligand	Equilibria	pK_f (Literature)	pK_f (Experimental)
8-hydroxyquinoline	$Ox^- + Mn^{2+} \rightleftharpoons MnOx^+$	6.8	6.51
Tiron (1,2-dihydroxy-benzene-3,5-disulfonic acid disodium salt	$HL^{3-} + Mn^{2+} \rightleftharpoons MnHL^-$	no value	4.09
HPO_4^{-2}	$HPO_4^- + Mn^{2+} \rightleftharpoons MnHPO_4$	2.58	2.67
EDTA (Ethylenediamine-tetraacetic acid	$Y^{4-} + Mn^{2+} \rightleftharpoons MnY^{2-}$	13.19	13.43

Formation Constant Values for Simple Ligands

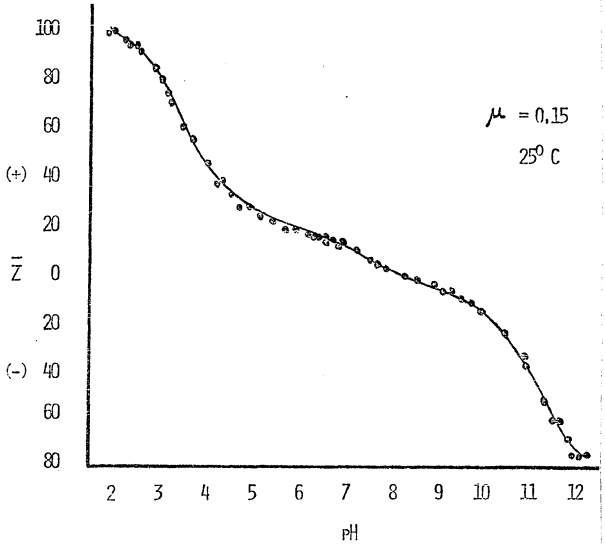
Determined by ESR Studies

TABLE 8

Because the acid dissociation characteristics of PAA have been presented in Table 2 of Section 1 and, because similar data have been determined by Tanford (1955) for BSA, Figure 12 and Table 9, only the quantity of manganous ion bound as a function of pH for each polymer is required to generate the respective K_{Int} values.

Figure 13 indicates that at a formal Mn(11) concentration of 2.5×10^{-4} M/L, 4.2×10^{-6} M/L of PAA binds a maximum of fifty metal ions (n=50). Table 10 presents typical data employed in the calculation of K_{Int} for Mn-PAA. The average intrinsic formation-constant was determined to be 1.4×10^{3} ($pK_{Int} = 3.15$), from sixteen experiments.

Guilbault and Meisel (1969) have shown that organic anions, which form ion-pair associations such as formate and acetate species, have no influence on the ESR behavior of Mn(11). By anology, this result might also be expected for β-carboxylate anions present on the PAA molecule. However, because bonding is seen to occur as a function of pH (Figure 13), it must be concluded that complexation occurs through the formation of a six-membered chelate involving an amide nitrogen and a carboxylic group to the extent of 25 % occupancy of all available binding sites. Takesada and Yamazaki (1966) postulated a similar phenomenon to explain the interactions between poly-glutamic acid and



BOVINE SERUM ALBUMIN TITRATION CURVE;
TANFORD, 1955

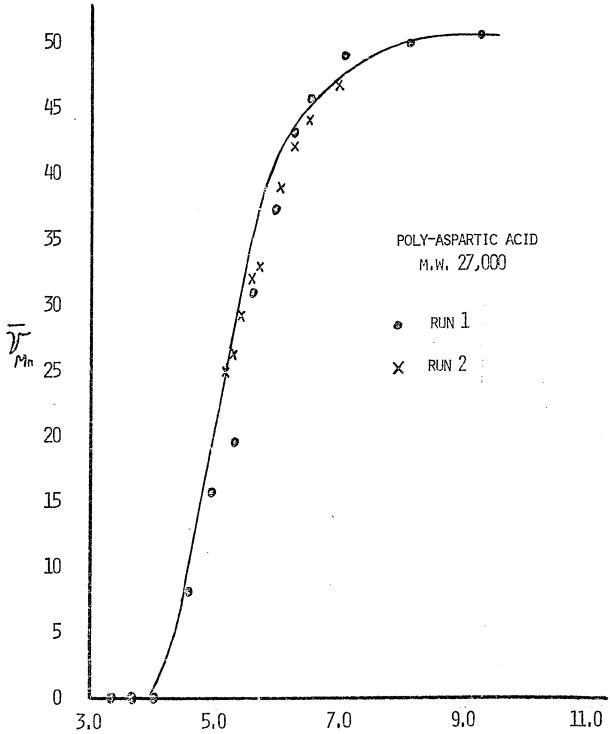
FIGURE 12

Ionizable Groups	$(pK_{Int})^{*}_{Diss}$
BSA-Carboxylic Acids	3.95
$HOOC-(CH_2)-COOH$	4.52
BSA-Phenols	10.35
Phenol	9.78

* Tanford, 1967.

The Intrinsic Acid Dissociation Constants of Bovine Serum Albumin Compared to Small Molecules

TABLE 9



THE BINDING OF MN(II) BY POLY-L-ASPARTIC AS A FUNCTION OF pH

FIGURE 13

Poly-L-Aspartic Acid

pH	α_H	$\bar{Z}$	$\bar{\nu}_{Mn}$	$\bar{Z}^*$	$(Mn)_{free}$	$(pK_{Int})_{Mn}$
4.861	0.400	-77.3	15.1	-47.1	1.87×10^{-4}	3.01
4.896	0.410	-79.2	14.4	-50.4	1.90×10^{-4}	2.92
5.040	0.450	-86.9	17.2	-49.3	1.51×10^{-4}	3.08

Bovine Serum Albumin

pH	α_H	$\bar{Z}$	$\bar{\nu}_{Mn}$	$\bar{Z}^*$	$(Mn)_{free}$	$(pK_{Int})_{Mn}$
7.955	0.515	-18.1	2.09	-13.9	1.75×10^{-4}	1.82
8.202	0.786	-22.1	3.17	-15.8	1.61×10^{-4}	1.86
9.000	0.890	-27.7	4.75	-18.2	1.39×10^{-4}	1.87

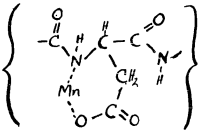
$\bar{Z}$ = polymer charge uncorrected for bound metal ions

$\bar{Z}^*$ = polymer charge corrected for bound metal ions

Representative Data Employed to Determine the Intrinsic Formation Constants of Mn(II) with Bovine Serum Albumin and Poly-Aspartic Acid

TABLE 10

Cu(11).



Similar calculations utilizing the data of Table 10 yield a value of 1.89 for the pK_{Int} of the Mn(11)-BSA association. The quantity (n) was not determined in this study because Gurd and Goodman (1952) have shown that only the 16 imidazole groups present on this molecule enter into complexation. Such a situation might, at first, appear to negate those conclusions drawn concerning the complexation ability of PAA because BSA is known to possess may glutamic and aspartic acid residues. One possible explaination is that in the pH range studied (6-8), the acidic carboxylic acid groups have complerely dissociated and have formed intra-molecular hydrogen bonds which maintain the overall globular structure of the polymer. Such an arrangement would hinder the interactions between the metal ion and the binding groups. evidence to support this thesis is of two forms: A pH of 9.7 must be obtained before any expansion of BSA occurs. This could imply phenolate-carboxylate repulsion. In addition, the added stability made available to the molecule by hydrogen bonding should be reflected in an increase of the acidic nature of the COOH groups involved as in the case of salicyclic acid. Table 9 indicates that such is the case.